

RESEARCH ARTICLE

Analysis of intraspecific variation in venoms of *Acanthophis antarcticus* death adders from South Australia

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ABSTRACT

Intraspecific variation in venom composition and activity has been reported from a wide range of snakes. Geographical origin can be one cause for this variation and has recently been documented from *Acanthophis antarcticus* death adders sampled across four different Australian states. The present study examined whether a narrower sampling range of *A. antarcticus* from four collection sites within one Australian state (*i.e.*, South Australia) would also exhibit variation in venom composition and/or activity. The present LC-MS results reveal marked differences in the venom composition from different collection sites. The most striking difference was the reduced venom complexity found in the only venom originating from a mallee scrub habitat in comparison to the venoms from coastal heath scrub habitats. Interestingly, the pharmacological activity of all venoms was found to be the same, independent of the collection site.

KEYWORDS: Death adder, *Acanthophis antarcticus*, venom, geographical and intraspecific variation, chick biventer cervicis nerve-muscle preparation

INTRODUCTION

Several studies have already reported intraspecific variation in snake venom composition and/or activity across a range of different snake species such as Western diamondback rattlesnakes *Crotalus atrox* (Minton and Weinstein, 1986), Tiger snakes *Notechis ater niger*, *N. scutatus* (Williams et al, 1988), *N. scutatus scutatus* (Yang et al, 1991), and Malayan pit vipers *Calloselasma rhodostoma* (Daltry et al, 1996). A range of factors have been implicated in the cause for intraspecific variation such as geographical origin (Glenn and Straight, 1978; Glenn et al, 1983), intersexual variation (Daltry et al, 1996), the snakes' diet (Gregory-Dwyer et al, 1986; Williams et al, 1988), ontogenetic development (Fiério et al, 1972), the frequency of milking (Willemse et al, 1979), seasonal effects (Bertrand and Vladesco, 1942), and genetically inherited individual variation (Gregory-Dwyer et al,

1986). Depending on the species of snake, one or several of these factors could have a greater impact than others.

Some recent studies found both inter- and intraspecific variation in the composition and activity of venoms from death adders (genus *Acanthophis*) belonging to the family Elapidae. Fry et al (2001) found that venoms from different geographical populations of the common death adder *A. antarcticus* differed not only in venom composition, but also in their neurotoxic activity and susceptibility to antivenom. In a subsequent study, venoms from several *Acanthophis* species and also from four regional Australian variants of *A. antarcticus* were fingerprinted by means of electrospray LC-MS (Fry et al, 2002). Specimens of *A. antarcticus* for the latter study were collected on a continent-wide geographic scale, specifically from populations in New South Wales, Queensland, South Australia and Western Australia. It was

found that less than 30% of the compounds were unique to any of the *A. antarcticus* venoms. Based on a parsimony analysis of all peaks, the *A. antarcticus* variants clustered together to form a single species that was distinct from the other *Acanthopis* species examined. On the other hand, the number of peaks and the variations in the detected masses also indicate clear differences between the *A. antarcticus* populations, possibly accounting for the observed differences in activity.

The present study examines whether similar intraspecific variation in venom composition and activity is observed within snakes sampled from a much narrower geographical range. Therefore, *A. antarcticus* were collected from four different sites within 600km distance of each other in South Australia. Their venom composition was analysed by means of RP-HPLC and LC-MS and their activity was assayed in the chick biventer nerve-muscle preparation.

MATERIALS AND METHODS

Snake collection and venom preparation

All *A. antarcticus* were wild-caught from four different sites in South Australia (see Figure 1). These snake populations were sourced from either coastal heath scrub habitats (*i.e.*, Smoky Bay, Elliston, and Tiddy Widdy Beach) or from a mallee scrub habitat (*i.e.*, Middleback Ranges). Their venom was then collected, freeze-dried and pooled according to the collection site by Venom Supplies Pty Ltd (Tanunda, SA, Australia), who provided the venom for this study. The source of each snake venom pool was as follows: Smoky Bay (two males and two female), Elliston (two males and one female), Tiddy Widdy Beach (one male and two females) and Middleback Ranges (two males).

Reverse-phase high performance liquid chromatography (RP-HPLC)

For HPLC 100µg of dried *A. antarcticus* venom from each collection site was fractionated using an analytical reverse-phase column (Jupiter 5µm, C₁₈, 150x2mm, Phenomenex Australia PTY Ltd, Sydney, Australia) connected to a Shimadzu HPLC system (10A series). Peptide peaks were monitored at an absorbance of 214nm and the flow rate was 0.2ml/min. Different linear gradients of eluent A (0.1%, v/v, trifluoroacetic acid (TFA)) and eluent B (90%, v/v, acetonitrile (ACN) + 0.9%, v/v, TFA) were used. During the first 5

min the percentage of eluent B was increased from 0–20% (= 0–18%, v/v, ACN), followed by an increase in eluent B from 20–60% (=18–54%, v/v, ACN) for 55min and a final increase from 60–80% eluent B (=54–72%, v/v, ACN) for 10min.

Liquid chromatography mass spectrometry (LC-MS)

Venom samples were analysed by LC-MS using a HCT ULTRA ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) coupled online with a 1200 series capillary HPLC (Agilent technologies, Santa Clara, CA, USA). Samples were injected onto a Zorbax 300SB reverse-phase column (5µm, C₁₈, 150x0.3mm) with solvent A (5%, v/v, ACN, 0.1%, v/v, formic acid) at a flow rate of 0.3µl/minute and kept at solvent A for 5min. Then a linear gradient of 0–90% of solvent B (90%, v/v, ACN, 0.1%, v/v, formic acid) was run for 30min. The eluant was nebulised and ionised using the Bruker electrospray source with the low flow electrospray needle and a capillary voltage of 4000V, dry gas at 300°C, a flow rate of 8l/min and nebuliser gas pressure at 1500mbar. The spectra were extracted and deconvoluted using Data Explorer software version 3.4 build 192 (Bruker Daltonics, Bremen, Germany).

Isolated chick biventer cervicis nerve-muscle preparation

Vertebrate toxicity was determined by the use of an isolated chick biventer cervicis nerve-muscle preparation. The muscles were removed from male chicks (8–15 days old), mounted in 5ml organ baths and maintained at 34°C under 1gm resting tension in a physiological saline solution of the following composition (in mM): 118.4 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂, 25 NaHCO₃, 11.1 D-glucose and bubbled continuously with 95%, v/v, O₂ + 5%, v/v, CO₂. Isometric contractions were measured via a Grass transducer (FTO3) connected to a Powerlab 4/20 system (ADInstruments, Australia). Twitches were evoked by stimulating the motor nerve (supramaximal voltage in the range of 12–20V depending on the tissue, 0.2ms, 0.1Hz) via silver electrodes connected to a Grass S88 stimulator. Nerve-mediated (indirectly evoked) twitches were confirmed by the addition of (+)-tubocurarine (10µM). In the absence of electrical stimulation, responses to exogenous acetylcholine (ACh) (1mM, 30sec), carbachol (0.02mM, 60sec) and KCl (40mM, 30sec) were obtained prior to the addition of venom and at the conclusion of the experiment. Preparations were allowed to equilibrate for at least 20min with continuous stimulation before the addition of venom.

Drugs and chemicals

Acetylcholine chloride, carbamylcholine chloride (carbachol), D-tubocurarine chloride were obtained from Sigma Chemical Co (St Louis, MO, USA). Stock solutions of drugs were made up in milliQ water unless otherwise stated.

Statistical analysis

For analysis of chick biventer data, Sigma-Stat 3.5 (Systat Software Inc., Chicago, USA) was used and values of $p < 0.05$ were considered significant. All data are expressed as mean ± standard error of the mean (SEM). Kruskal-Wallis ANOVA was used to determine the effects of the pooled venoms on the twitch height in the chick biventer

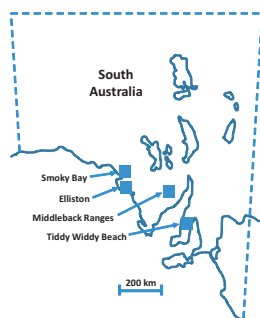


Figure 1. Distribution of the four *A. antarcticus* collection sites within South Australia (state borders indicated by the interrupted line). The sites were Smoky Bay, Elliston, Middleback Ranges and Tiddy Widdy Beach.

cervicis assay followed by Dunn's Method for pairwise multiple post-hoc comparisons. Mann-Whitney rank sum tests were used to compare the responses of the isolated chick biventer muscles to acetylcholine, carbachol and KCl before and after addition of the venom.

For analysis of the LC-MS data, the deconvoluted masses from all venom pools were compared against each other. Masses from different venom pools were considered to be identical when falling into the same mass ± 1 Da and retention time ± 1 min window. A clustering analysis for those with similar masses was carried out using JMP (version 3.2.6, SAS Institute, Cary, USA). All other masses were considered to be unique for each venom pool.

RESULTS AND DISCUSSION

The great majority of peaks were found across the HPLC chromatograms of venoms from all collection sites (Figure 2). Although some of the major peaks appear to be

shifted between venoms from different collection sites, this can be attributed to variability in the retention times for different runs. We have noticed that for repeated runs of venom from the same collection site (data not shown), the retention times especially for the first few peaks eluting within 20min shifted considerably, sometimes by up to 4min. This may be explained by differing column temperatures, as we did not have access to a column oven and the HPLC was located in a room with variable temperatures due to sunlight exposure, depending on the time of day. For all peaks eluting after 20min, the variation was usually within 1min. The peak heights between different collection sites were also found to vary considerably, indicating different expression levels of similar toxins between different collection sites. Although the dried venom amount used for HPLC from each collection site was always the same, the trace from Middleback Ranges seems to show a generally lower level of absorbance across all peaks as compared to venom from the other collection sites. One explanation could be a higher content of venom components not absorbing at 214nm. In addition,

RP-HPLC of *A. antarcticus* venoms

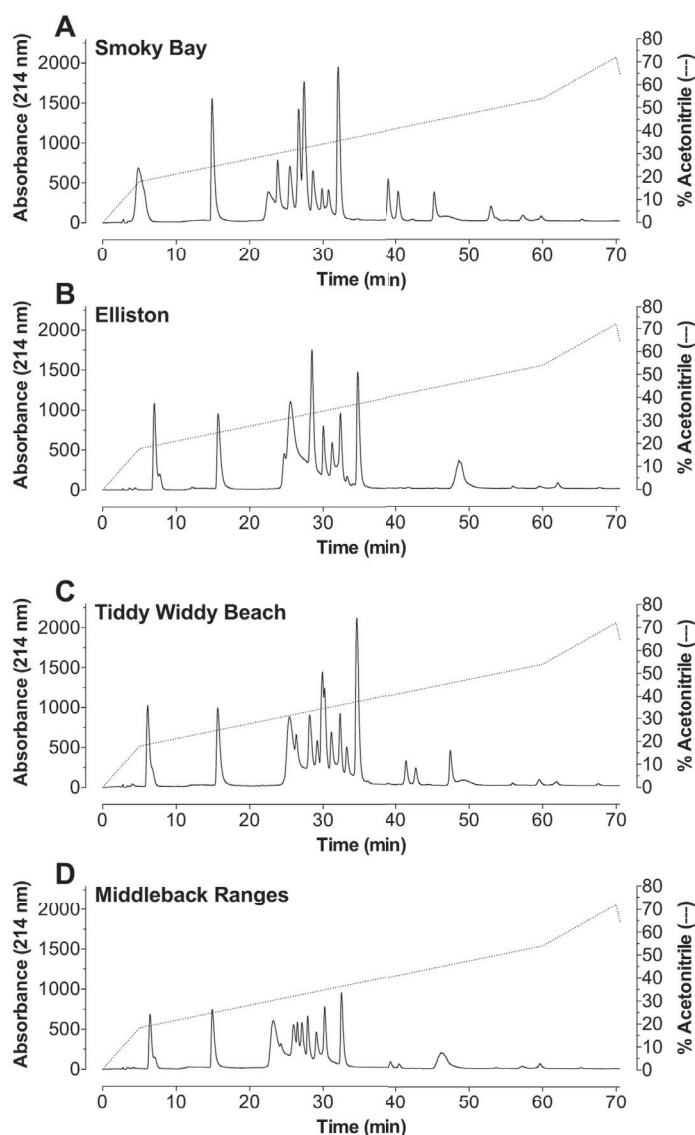


Figure 2. RP-HPLC chromatograms of *A. antarcticus* venoms from four different South Australian collection sites (A-D). Note that no peaks were observed between 70min and 80min retention time, therefore this time-window is not indicated.

two subsequent peaks eluting around 40min were observed in venom from all collection sites with the exception of Elliston (see Figure 2B), which marked the most outstanding geographical variation observed by means of RP-HPLC. Based on a comparison with the LC-MS traces in Fry et al (2002), those late eluting components could be either similar to phospholipase A₂ or taipoxin gamma chain, as the other components such as natriuretic peptides, Kunitz-type proteases or short and long chain α -neurotoxins should elute earlier.

Analysis of the LC-MS data on the other hand, revealed more considerable differences in venom composition between all collection sites. The majority of all masses from all collection sites were actually unique to each site (Table 1), with the highest percentage of unique masses being present in the Smoky Bay venom (85.6%), followed by Tiddy Widdy Beach (81.1%), Elliston (77.3%) and Middleback Ranges (50%). Given that a previous study found less than 30% of unique masses from *A. antarcticus* populations from all over Australia (Fry et al, 2002), the results of the present study may be explained by the more stringent criteria used to determine the masses that are identical between venoms. We used a window of ± 1 Da and ± 1 min to determine identical masses, whereas Fry et al (2002) used a window of ± 2 Da without any restriction on the retention time. However, when assuming that the 'same' component is present in different venoms that show the presence of identical masses, this component should then also elute at a similar time on the LC run. If this is not the case, then the mass might just be coincidentally identical, while the sequence of the component could be completely different. Furthermore, for *Acanthophis* venoms in general, the previous study concluded a minimal level of conservation of individual molecules (Fry et al, 2002), which is in line with our finding of the majority of masses being unique to each collection site of *A. antarcticus*.

The same order that was found for the number of unique masses was also observed for the total number of masses, with 77 masses for both Smoky Bay and Tiddy Widdy Beach, 66 masses for Elliston and only 24 masses for Middleback Ranges (50%). These results are quite interesting, as the Middleback Ranges collection site (which has the lowest number in total and in unique venom masses) was the only mallee scrub habitat, whereas the other three venoms originated from coastal heath scrub habitats. This could indicate that a coastal heath scrub habitat favours a more complex venom composition, whereas mallee scrub habitats favour a less complex venom with the highest percentage of masses identical to masses found in venoms from other collection sites. The venom from Middleback Ranges could even be viewed as being reduced to a minimum composition required for activity, as its' activity in the chick biventer was found to be similar to venom from the other collection sites (see below). However, this conclusion has to be taken with caution, as chicken are not among the natural prey of *A. antarcticus*. The reasons for the reduced complexity observed in the venoms from the mallee scrub habitat are yet unknown. It could, for example, be due to quantitative and qualitative differences in the availability of prey. Another explanation could be that mallee scrub habitats are considered semi-arid, whereas more rainfall is expected on

the coastal heath scrub habitats. As a way to conserve water and energy resources, the venoms in the harsher mallee scrub habitat could be reduced in their complexity. Further research is required to determine the reason for the lower venom complexity in mallee scrub habitats.

The results for the clustering analysis (Figure 4) of identical masses between different collection sites (based on data from Table 2) were rather surprising. Based on the geographical proximity of Smoky Bay and Elliston, one could have assumed that those venoms would show the closest similarity, whereas the Tiddy Widdy Beach and Middleback Ranges venom could be more similar to each other (Figure 1). However, we found that the composition of the Tiddy Widdy Beach and Elliston venoms were most closely related, as were the venoms from Middleback Ranges and Smoky Bay. The clustering results are also not explained by the different habitats, as the Middleback Ranges venom would have been expected to be separate from the other three venoms. Furthermore, the fact that the venom from Middleback Ranges was only sourced from male *A. antarcticus*, whereas all other pools are composed of venom from both male and female snakes, could point to an intersexual variation in *A. antarcticus* venom composition. While the reduced complexity of venom from Middleback Ranges might be partially explained by the lack of female *A. antarcticus* venom in the pool, the clustering analysis does not support a strong involvement of intersexual variation, as the two samples with the highest content of male venom, (i.e., Middleback Ranges and Elliston) would be expected to form a cluster, which was not the case. In conclusion, the present results could point to a common ancestor for the *A. antarcticus* populations in Smoky Bay and Middleback Ranges (the same applies to the populations from Tiddy Widdy Beach and Elliston). Based on the present results, it cannot be determined whether the venom complexity in Middleback Ranges was reduced after the snakes inhabited this area or if their common ancestor already had a reduced venom complexity, which was then increased for the Smoky Bay population after inhabiting the coastal heath scrub environment.

Analysis of the twitch height data from the chick biventer experiments (Figure 3A) revealed that all venom pools differed significantly from the control group (all $P < 0.05$; control vs. Smoky Bay $Q=13.511$, control vs Elliston $Q=14.651$, control vs Tiddy Widdy Beach $Q=10.823$, control vs Middleback Ranges $Q=12.888$). Additionally, venom from Elliston showed a significant difference in the twitch height response to venom from Tiddy Widdy Beach ($p < 0.05$, $Q=3.689$). The comparison of all the other venoms did not reveal any significant differences (all $p > 0.05$). This indicates that independent of the collection site, all venoms caused a significant inhibition of the electrically-induced muscle contractions with the Elliston venom pool causing the quickest response.

In addition, the responses of the isolated chick biventer muscles to acetylcholine, carbachol and KCl, before and after the venom was added, were compared using Mann-Whitney rank sum tests. The contractile responses before and after addition of the venom revealed significant effects on the response to acetylcholine and carbachol in all snake

Table 1. Results for LC-MS analysis of venoms, indicating the molecular masses of the +1 ion (monoisotopic for masses up to 20kDa, average for anything >20kDa) and the respective retention times for all masses that were unique to each collection site.

Smoky Bay		Tiddy Widdy Beach		Elliston		Middleback Ranges	
Mol mass (Da)	Ret time (min)	Mol mass (Da)	Ret time (min)	Mol mass (Da)	Ret time (min)	Mol mass (Da)	Ret time (min)
746.2	25.2–25.6	615.3	33.2–33.5	739.8	27.3–27.7	744.9	28.8–29.1
801.8	29.4–29.7	834.9	27.4–27.7	822.4	27.3–27.7	909.9	28.8–29.1
819.2	29.4–29.7	909.9	25.3–25.8	823.7	27.3–27.7	958.9	27.6–27.8
859.9	25.2–25.6	910.0	28.7–28.9	910.4	25.3–25.9	1023.5	28.8–29.1
885.0	25.2–25.6	913.3	25.3–25.8	1023.5	25.3–25.9	1433.3	28.8–29.1
886.5	25.2–25.6	954.7	28.7–28.9	1029.4	25.3–25.9	1667.3	27.6–27.8
890.4	25.2–25.6	955.9	27.4–27.7	1029.7	28.8–28.9	2053.7	27.6–27.8
902.9	29.4–29.7	975.8	27.4–27.7	1037.4	25.3–25.9	2856.8	27.6–27.8
959.1	25.2–25.6	1031.7	28.7–28.9	1169.5	27.3–27.7	3091.6	28.8–29.1
986.0	25.2–25.6	1031.8	28.8–28.9	1173.9	27.3–27.7	3325.5	27.6–27.8
1118.8	25.2–25.6	1111.3	27.4–27.7	1178.4	27.3–27.7	3867.4	28.8–29.1
1178.5	25.2–25.6	1478.5	27.4–27.7	1183.0	27.3–27.7	4124.2	27.6–27.8
1331.2	27.5–27.7	1636.8	33.2–33.5	1183.4	27.3–27.7		
1663.0	29.4–29.7	1664.0	27.4–27.7	1185.5	25.3–25.9		
1779.8	25.2–25.6	1671.4	27.4–27.7	1637.0	25.3–25.9		
1825.0	28.8–29.1	1674.5	27.4–27.7	1642.7	27.3–27.7		
1834.2	29.4–29.7	1677.6	25.3–25.8	1643.1	25.3–25.9		
1943.3	29.4–29.7	1818.3	27.4–27.7	1681.7	27.3–27.7		
2024.2	25.2–25.6	1829.7	28.7–28.9	1833.8	25.3–25.9		
2220.7	29.4–29.7	1926.5	27.4–27.7	1916.3	25.3–25.9		
2225.4	28.8–29.1	2045.4	27.4–27.7	1916.4	27.3–27.7		
2339.7	25.2–25.6	2062.5	28.7–28.9	1963.4	27.3–27.7		
2345.9	28.8–29.1	2218.4	27.4–27.7	2063.0	25.3–25.9		
2405.2	28.8–29.1	2227.5	27.4–27.7	2063.8	27.3–27.7		
2405.5	29.4–29.7	2227.7	27.4–27.7	2075.1	27.3–27.7		
2416.6	29.4–29.7	2231.2	28.7–28.9	2222.3	27.3–27.7		
2454.9	25.2–25.6	2236.1	25.3–25.8	2224.7	25.3–25.9		
2469.3	28.8–29.1	2332.8	33.2–33.5	2358.9	27.3–27.7		
2476.2	25.2–25.6	2337.7	27.4–27.7	2368.2	25.3–25.9		
2503.8	28.8–29.1	2365.9	25.3–25.8	2369.5	27.3–27.7		
2579.0	29.4–29.7	2469.0	33.2–33.5	2454.4	27.3–27.7		
2710.9	29.4–29.7	2509.5	27.4–27.7	2468.7	25.3–25.9		
2715.4	29.4–29.7	2516.0	27.4–27.7	2514.6	27.3–27.7		
2724.7	29.4–29.7	2542.8	33.2–33.5	2760.1	27.3–27.7		
2738.5	29.4–29.7	2580.5	28.8–28.9	2850.6	27.3–27.7		
2749.7	25.2–25.6	2724.4	33.2–33.5	3076.8	27.3–27.7		
2758.0	25.2–25.6	2858.8	33.2–33.5	3093.5	27.3–27.7		
2765.9	25.2–25.6	2867.7	28.7–28.9	3331.0	27.3–27.7		
2864.9	29.4–29.7	2867.7	28.8–28.9	3635.6	27.3–27.7		
2945.1	25.2–25.6	2876.4	27.4–27.7	4090.4	27.3–27.7		
3029.5	25.2–25.6	2881.6	27.4–27.7	4141.1	27.3–27.7		
3049.2	25.2–25.6	2898.1	27.4–27.7	4700.5	27.3–27.7		
3083.4	29.4–29.7	3028.1	33.2–33.5	7165.3	28.8–28.9		
3098.9	29.4–29.7	3066.6	27.4–27.7	8211.2	28.8–28.9		
3503.0	29.4–29.7	3067.2	33.2–33.5	8215.3	28.8–28.9		
3507.7	29.4–29.7	3085.3	33.2–33.5	16455.0	28.8–28.9		
3631.5	28.8–29.1	3086.7	28.8–28.9	19162.6	28.8–28.9		
3632.6	29.4–29.7	3090.6	33.2–33.5	31093.0	28.8–28.9		
3656.8	28.8–29.1	3091.7	25.3–25.8	32945.7	28.8–28.9		
3869.7	28.8–29.1	3104.8	25.3–25.8	39981.9	28.8–28.9		
3877.1	28.8–29.1	3112.4	25.3–25.8	80943.4	28.8–28.9		
3882.5	28.8–29.1	3222.9	28.7–28.9				
3885.8	29.4–29.7	3327.6	27.4–27.7				
4036.4	25.2–25.6	3347.2	27.4–27.7				
4089.8	29.4–29.7	3363.3	27.4–27.7				

(continued)

Table 1. Continued

Smoky Bay		Tiddy Widdy Beach		Elliston		Middleback Ranges	
Mol mass (Da)	Ret time (min)	Mol mass (Da)	Ret time (min)	Mol mass (Da)	Ret time (min)	Mol mass (Da)	Ret time (min)
4106.5	29.4–29.7	3513.1	28.7–28.9				
4306.5	28.8–29.1	3513.6	25.3–25.8				
4312.0	28.8–29.1	3526.1	27.4–27.7				
4833.0	28.8–29.1	3526.4	33.2–33.5				
6650.4	27.5–27.7	3526.7	28.7–28.9				
6667.3	27.5–27.7	3536.0	25.3–25.8				
21051.9	27.5–27.7	3548.5	25.3–25.8				
24753.5	27.5–27.7	3568.2	25.3–25.8				
27643.6	28.8–29.1	4105.7	27.4–27.7				
34807.8	28.8–29.1	4111.9	27.4–27.7				
34937.4	27.5–27.7	4122.0	33.2–33.5				
36664.2	27.5–27.7	4276.1	28.8–28.9				
42997.0	28.8–29.1	4276.8	28.7–28.9				
49232.6	28.8–29.1	8596.3	28.8–28.9				
51417.1	28.8–29.1	12894.7	28.8–28.9				
51478.6	27.5–27.7	16426.4	28.8–28.9				
57207.5	27.5–27.7	19980.6	28.8–28.9				
57304.6	28.8–29.1	49911.5	28.8–28.9				
57709.3	28.8–29.1	51501.1	28.8–28.9				
59238.6	27.5–27.7	58521.6	28.8–28.9				
70109.4	28.8–29.1	62731.4	28.8–28.9				
71601.4	28.8–29.1	77605.4	28.8–28.9				

Table 2. Results for LC-MS analysis of venoms, indicating the monoisotopic molecular masses of the +1 ion and the respective retention times for all masses that were found in two or more venoms from different collection sites.

Molecular mass (Da)	Smoky Bay	Tiddy Widdy Beach	Elliston	Middleback Ranges
832.3		27.4–27.7	27.3–27.7	
839.4	25.2–25.6			25.3–25.8
913.5		28.7–28.9	27.3–27.7	
915.2			25.3–25.9	25.3–25.8
951.0		27.4–27.7		27.6–27.8
953.7		27.4–27.7	27.3–27.7	
1176.3		25.3–25.8	25.3–25.9	
1825.5		27.4–27.7	27.3–27.7	
1829.4	25.2–25.6	25.3–25.8		
1915.3	27.5–27.7	27.4–27.7		
2058.1		28.8–28.9		28.8–29.1
2217.0			27.3–27.7	27.6–27.8
2357.2	25.2–25.6		25.3–25.9	
2454.9	29.4–29.7	28.7–28.9		
2468.7		28.7–28.9	28.8–28.9	28.8–29.1
2499.1		27.4–27.7	27.3–27.7	
2579.8	28.8–29.1	28.7–28.9		
2737.8	25.2–25.6			25.3–25.8
2743.7		28.7–28.9	27.3–27.7	
2869.4	28.8–29.1	27.4–27.7		
3085.0		28.7–28.9		27.6–27.8
3086.0	25.2–25.6			25.3–25.8
3093.4	25.2–25.6			25.3–25.8
3106.0	25.2–25.6		25.3–25.9	
3352.8		27.4–27.7	27.3–27.7	
3505.8			27.3–27.7	27.6–27.8
3526.7	25.2–25.6			25.3–25.8
8180.5	28.8–29.1	28.8–28.9	28.8–28.9	

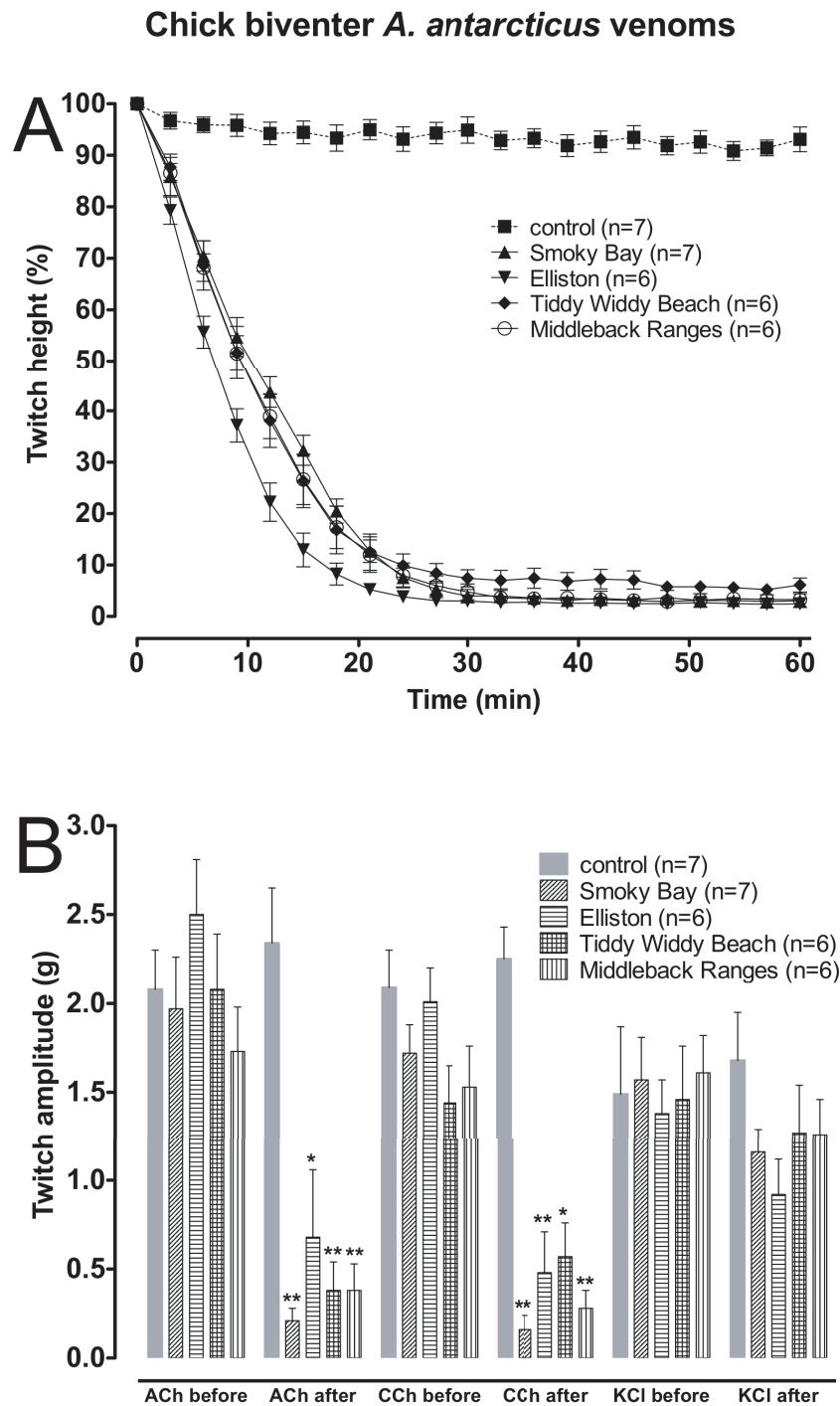


Figure 3. A. Twitch height as measured in the chick biventer model after 3µg/ml of *A. antarcticus* snake venom from four different collection sites within South Australia. 10µl water/ml was used in the control group. **B.** Twitch amplitude in the same muscles as a response to acetylcholine (ACh), carbachol (CCh) and potassium chloride (KCl) before and after the venom was added. *($p<0.05$) and **($p<0.01$) indicate significant differences by comparison of the twitch amplitude before vs after. The number of chick biventer muscles used for each group is indicated (n=6-7).

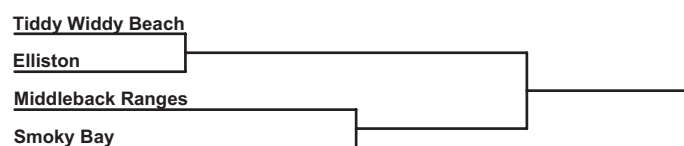


Figure 4. Clustering results based on the LC-MS data for molecular masses that were found in venoms from two or more collection sites (see Table 2).

venom treated groups (all $p < 0.05$ or $p < 0.01$, see Figure 3B), but not in the control group. The responses to KCl were unaltered by all of the venoms and by the effects of time (all $p > 0.05$). These results indicate that all venoms caused a postsynaptic block of neuromuscular transmission, which is consistent with activity of various postsynaptic neurotoxins reported from the venom of *A. antarcticus* (for an overview see Van der Weyden et al, 1997).

CONCLUSIONS

Overall, the present RP-HPLC results indicate only minor geographical variation between venoms from different collection sites of *A. antarcticus*. On the other hand, the LC-MS results reveal considerable differences in venom composition, with the most striking being the reduced complexity of venom sourced from a mallee scrub habitat, as compared to the venoms sourced from coastal heath scrub habitats. Despite these marked differences in venom composition, all venoms were similarly active in the chick biventer assay. The present results of four *A. antarcticus* venoms sampled from a narrow geographical range within South Australia therefore reflect the variation in venom composition but not in activity that was previously observed with *A. antarcticus* venoms sampled from a much larger geographical range (Fry et al, 2002).

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COMPETING INTERESTS

None declared.

LIST OF ABBREVIATIONS

ACh; Acetylcholine
ACN; Acetonitrile
CCh; Carbachol
KCl; Potassium chloride

LC-MS; Liquid-chromatography followed by mass spectrometry
RP-HPLC; Reverse-phase high performance liquid chromatography

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